STANDARD CURVE OF GFP FLUORESCENCE

1. Wash quartz cuvette twice with 0.1M NaOH and twice with 0.1M HCl, followed by 10 washes with double distilled water.

2. Add 1mL of protein solution to previously washed and dried quartz cuvette

3. Select in fluorimeter excitation wavelength of 488nm, emission wavelength of 509nm. Reading retard of 0s, with stirring at 37ºC. Readings in triplicate.

4. Start measurement and record results. Use protein buffer (generally lysis buffer) as blank.

5. Test following protein solutions as listed in table below, to generate a standard curve. All points should have 3 technical replicates, and 3 sample replicates.

[GFP]		Fluorescence / a.u.
µg/mL	nM	
1000	41,7	
750	31,25	
500	20,83	
100	4,17	
75	3,13	
50	2,08	
25	1,04	
15	0,63	
10	0,42	
0	0	

Make the linear regression, excluding the most discrepant points using Microsoft Excel software (or other similar calculation software):

R^2: _____ ; y = ___ x + (____)

6. After a measure, wash quartz cuvette as referred in step 1.